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ORIGINAL ARTICLE

Randomized comparative trial of mizoribine versus mycophenolate mofetil in combination with tacrolimus for living donor renal transplantation

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Abstract
Background Mizoribine (MZR) was approved in 1984 in Japan for the suppression of rejection in renal transplantation with an approved administration dosage of 1–3 mg/kg/day. The action of MZR resembles that of mycophenolate mofetil (MMF), but MZR dosing is markedly lower than that of MMF. To examine whether higher dosing of MZR could obtain efficacy similar to MMF in renal transplantation, we conducted a comparative study of MZR and MMF using a high daily dose of MZR.
Methods A prospective, randomized comparative study of MZR versus MMF using tacrolimus (FK) and steroids as the base was conducted in 35 patients who had undergone living-donor renal transplantation (ABO-incompatible patients were not included) at 8 institutions in Japan between July 2005 and June 2007. Starting doses were 12 mg/kg/day for MZR and 2 g/day for MMF. Dosages of FK and steroids were set according to the protocol of each institution.
Results Patient and graft survival rate at 1 year after transplantation was 100 % in each group, with no significant difference in rejection rate apparent between groups. Adverse events found in both groups were characteristic, frequently involving infection and digestive organ disorder in the MMF group and elevated uric acid levels in the MZR group.
Conclusions Based on these results, MZR and MMF are considered almost equivalent in terms of efficacy and safety.

Keywords Renal transplantation · Tacrolimus · Mycophenolate mofetil · Mizoribine · Randomized comparative study

Introduction

Administration of a calcineurin inhibitor (CNI) combined with steroids and a metabolic antagonist has been used as immunosuppressive therapy in patients undergoing renal transplantation. Each of the drugs used causes specific adverse reactions, but combined use of several drugs

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can minimize such reactions while yielding sufficient immunosuppressive effects. Immunosuppression in renal transplantation must be balanced against the risk of post-transplantation infection, so control of the immunosuppressed state is crucial. From this perspective, immunosuppressive therapy that causes less severe adverse reactions is clearly desirable.

Mizoribine (MZR) was discovered as an antifungal antibiotic produced by the ascomycete *Eupenicillium brefeldianum* M-2166, isolated from soil obtained on Hachijo island, Japan, in 1971 [1]. The mechanism of action of MZR is inhibition of inosine monophosphate dehydrogenase, the rate-limiting enzyme for de novo synthesis of purine in mammalian cells. The action of MZR resembles that of mycophenolate mofetil (MMF), although the inhibition pattern differs, involving selective suppression of lymphatic cell proliferation, and reduction of humoral and cellular immune responses [2].

The currently approved administration and dosage schedule of MZR is 1–3 mg/kg/day by oral administration once, or in divided doses to 2 or 3 times daily, markedly less than that of MMF. Therefore, if a sufficient amount of MZR can be administered, efficacy similar to that of MMF may be possible. To examine the safety of MZR at high doses (phase 1), a single-dose study using 3, 6, 9 or 12 mg/kg/day and a 7-day consecutive administration study of MZR at a daily dose of 12 mg/kg (divided doses of 6 mg/kg in the morning and afternoon) were conducted from February to April 2004 in Belfast, UK [3]. This study revealed hardly any adverse reactions attributable to MZR, indicating the safety of the regimen used. Hence, for the purposes of examining the utility of MZR at a daily dose of 12 mg/kg (divided doses of 6 mg/kg twice, in the morning and afternoon), we conducted a comparative study with MMF, the current international standard as a metabolic antagonist for renal transplant recipients.

Materials and methods

A prospective, randomized comparative study of MZR versus MMF using tacrolimus (FK) and steroids, but not basiliximab, as the base was conducted in 35 patients who underwent living-donor renal transplantation (ABO-incompatible patients were not included) at 8 institutions in Japan between July 2005 and June 2007. Starting doses were 12 mg/kg/day for MZR and 2 g/day for MMF. Dosages of FK and steroids were set according to the protocol of each institution.

After obtaining approval from the ethics committee of each participating institution, renal transplantation patients, from whom informed consent had been obtained after receiving an explanation of the purposes of the study, were registered on a designated website and assigned to the study groups. At the beginning, 17 patients were assigned to the MZR group and 19 patients to the MMF group. However, 1 patient in the MZR group withdrew consent before the start of administration, so 16 patients in the MRZ group and 19 patients in the MMF group (35 patients in total) were used for analysis. No inter-group differences were seen in patient background characteristics such as underlying disease in recipient, sex, age, body weight, dialysis period or number of HLA-incompatible cases (Table 1). As a rule, acute rejection was judged according to the Banff classification (1997) after conducting a renal biopsy. The main endpoint was the rejection-free rate at 1 year after transplantation, and secondary endpoints were patient survival rate, graft survival rate and incidence of adverse events during the first year after transplantation.

Student's t test, the χ^2 test and the log-rank test were used for statistical analyses.

Results

FK and steroids were administered according to the protocol of each institution. The dose of FK in the early stage after transplantation was 0.08–0.12 mg/kg/day, and trough concentrations in the early stage and from 3 months after transplantation onward were adjusted to 10–15 and 5–10 ng/ml, respectively. With regard to steroids, methylprednisolone was administered intravenously at a dose of 0.5–1 g/day for 3 days after transplantation. Thereafter, a steroid was administered orally at 30–40 mg/day (prednisolone equivalence). The dose was gradually reduced to less than 10 mg/day after 2–3 months. In terms of the trough concentration of FK and the dose of steroids, no significant differences were found between the MZR and MMF groups. Administration of MZR was started at an average dose of 600 mg/day, divided into morning and afternoon doses. The dose was gradually reduced from 2 months onward, with a mean dose after 1 year of 350 mg/day. Administration of MMF was started at an average dose of 2 g/day, divided into morning and afternoon doses. The dose was gradually reduced from 2 weeks onward, with a mean dose after 1 year of 1 g/day (Fig. 1).

At 1 year after transplantation, patient and graft survival rates were 100 % in both groups. Freedom from acute rejection is shown in Fig. 2, and the results of biopsy for identification of acute rejection are shown in Table 2. The acute rejection-free rate at 1 year after transplantation was 56.2 % in the MZR group and 47.4 % in the MMF group, with no significant difference between groups. The incidence of acute rejection other than borderline change was 25.0 % in the MZR group and 21.1 % in the MMF group. Examination of rejection severity by biopsy demonstrated:

Table 1 Demographics and baseline characteristics

	MZR ($n = 16$)	MMF ($n = 19$)	P
Cause of uremia (%)			
Chronic glomerulonephritis	9 (56)	10 (53)	0.549
Diabetic nephropathy	0 (0)	3 (16)	
Focal glomerulosclerosis	0 (0)	1 (5)	
Membranoproliferative glomerulonephritis	1 (6)	0 (0)	
Others	6 (38)	5 (26)	
Recipient sex (%)			
Men	9 (56)	15 (79)	0.150
Women	7 (44)	4 (21)	
Recipient age (years)[a]	36.1 ± 7.2	39.7 ± 11.3	0.134
Recipient weight (kg)[a]	53.8 ± 9.4	61.1 ± 15.3	0.052
Duration of hemodialysis (years)[b]	1.5 (0–7.9)	1.6 (0–6.8)	0.608
Donor sex (%)			
Men	9 (56)	8 (42)	0.404
Women	7 (44)	11 (58)	
Donor age (years)[a]	57.6 ± 14.1	54.8 ± 12.7	0.724
Donor type (%)			
Father	6 (38)	5 (26)	0.529
Mother	7 (44)	6 (32)	
Sibling	2 (13)	5 (26)	
Others	1 (6)	3 (16)	
HLA-AB mismatches (%)			
0	2 (13)	3 (16)	0.176
1	2 (13)	6 (32)	
2	10 (63)	5 (26)	
3	1 (6)	3 (16)	
4	0 (0)	2 (11)	
Unknown	1 (6)	0 (0)	
HLA-DR mismatches (%)			
0	4 (25)	7 (37)	0.592
1	10 (63)	10 (53)	
2	1 (6)	2 (11)	
Unknown	1 (6)	0 (0)	
ABO blood type (%)			
Identical	11 (69)	10 (53)	0.332
Compatible	5 (31)	9 (47)	

[a] ± values indicate mean ± SD

[b] Median (range)

Banff 1A in 2 cases in the MZR group and 3 cases in the MMF group; Banff 1B in 1 case in the MZR group; and antibody-related rejection in 1 case in each of the 2 groups. None of these items differed significantly in incidence between groups. Serum creatinine levels at 1, 3, 6 and 12 months after transplantation were 1.66 ± 0.69, 1.39 ± 0.53, 1.35 ± 0.57 and 1.24 ± 0.47, respectively, in the MZR group, and 1.44 ± 0.42, 1.43 ± 0.46, 1.45 ± 0.59 and 1.39 ± 0.47, respectively, in the MMF group (Fig. 3). Again, no significant inter-group differences were evident.

Numbers of patients showing adverse events and numbers of adverse events were 13 of 16 patients (81.1 %) and 21 events in the MZR group, and 14 of 19 patients (73.7 %) and 25 events in the MMF group. The main adverse events observed are shown in Table 3. Elevated uric acid levels were found in 6 of 16 patients (37.5 %) in the MZR group and 4 of 19 (21.1 %) in the MMF group. Although the number of cases in the MZR group was higher, no significant inter-group difference was identified. Conversely, incidences of viral infection and digestive organ disorder were 42.1 % (8/19 cases) and 10.5 % (2/19

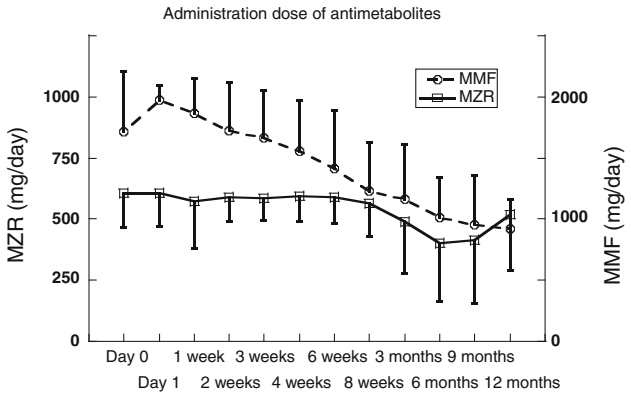

Fig. 1 Administration dose of antimetabolites

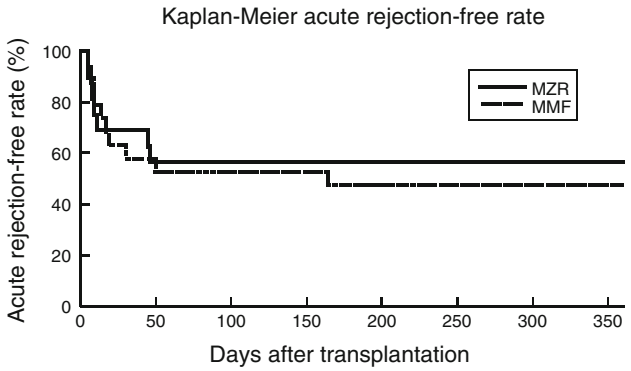

Fig. 2 Kaplan–Meier analysis of the acute rejection-free rate

Table 2 Acute rejection

	MZR group ($n = 16$)	MMF group ($n = 19$)
Normal	9 (56.2 %)	9 (47.4 %)
Borderline changes	3 (18.8 %)	6 (31.6 %)
Acute rejection	4 (25.0 %)	4 (21.1 %)
Banff 1A	2	3
Banff 1B	1	0
AMR susp.	1	1

cases), respectively, in the MMF group, compared with 25.0 % (4/16 cases) and 0 %, respectively, in the MZR group. Again, these inter-group differences were not significant. In addition, the presence or absence of antibody against cytomegalovirus (CMV) in donors and recipients was surveyed before transplantation. This revealed 4 combinations of donor antibody-positivity and recipient antibody-negativity in the MZR group. After excluding these 4 patients, rates of CMV infection in the 2 groups were 16.7 % (2/12 cases) in the MZR group and 36.8 % (7/19 cases) in the MMF group. However, as the number of

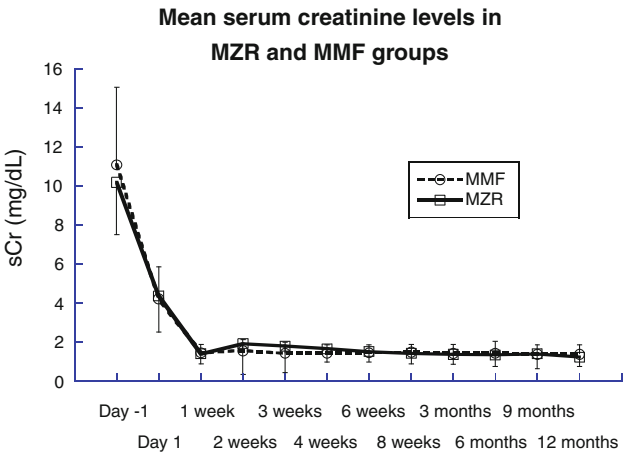

Fig. 3 Mean serum creatinine levels in the MZR and MMF groups

analyzed cases was small, this difference did not reach the level of significance (χ^2 test, $P = 0.228$).

Discussion

Cyclosporine or FK as CNIs are used in combination with steroids and metabolic antagonists for immunosuppressive therapy in renal transplant recipients. Reported CNI-specific adverse reactions have included nephropathy, diabetes, finger tremor, hyperlipidemia, cardiotoxicity, and hepatopathy. In the case of steroids, short stature and peptic ulcers in children, cataract–glaucoma, hypertension, and osteoporosis have been reported [4, 5]. In addition, in the case of metabolic antagonists, adverse reactions such as bone marrow suppression and hepatopathy induced by azathioprine (AZT), and digestive organ disorder induced by MMF are frequently observed [3, 6].

MZR was first approved in 1984 in Japan for the suppression of rejection in renal transplantation. Clinical trials of MZR for renal transplantation (phase III studies) were conducted from 1978 to 1982. At that time, however, CNIs had not been developed, the main therapeutic drugs were AZT and steroids [7], and the total number of renal transplants conducted annually nationwide in Japan was only 200–300. As a result of renal transplantation, the 1-year survival rate of kidney graft was about 65 % for living-donor transplantation and 20–30 % for cadaveric kidney transplantation. Since prevention and treatment of rejection were insufficient, renal function could not be restored in many cases at an early stage after transplantation, and a high serum creatinine level was observed. Since 100 % of MZR is excreted renally, this agent is effective even at daily doses no more than 3 mg/kg in patients whose renal function could not be fully restored at an early stage. Conversely, renal function was severely reduced in an

Table 3 Main adverse events and infections

	MZR group ($n = 16$)	MMF group ($n = 19$)	P
Blood			
Leukopenia	2	2	0.855
Anemia	1	2	0.653
Infections			
CMV	4	7	0.452
BKV	0	1	0.352
Elevation of serum uric acid	6	4	0.283
Digestive			
Diarrhea	0	1	0.352
Stomachache	0	1	0.352

appreciable proportion of patients after renal transplantation (serum Cr level no less than 4.0 mg/dl). For these patients, there was some apprehension about increasing the MZR dose further, given the possible development of adverse reactions such as bone marrow suppression. Therefore, at the time of MZR approval in 1984, the administration and dosage of MZR were set at 1–3 mg/kg/day orally in divided doses 1–3 times daily.

Cyclosporine was developed in the latter half of the 1980s and FK was developed in 1994. In addition, because of technical progress in transplantation medicine, the clinical results for renal transplantation improved substantially. Three types of drugs are currently employed for immunosuppressive therapy in renal transplantation: a CNI, a metabolic antagonist, and a steroid as the base. Recently, the 1-year graft-survival rate in Japan among renal transplant recipients achieved using these 3 types of drugs in combination has exceeded 90 % for living-donor transplantation and 80 % for cadaveric kidney transplantation. This progress in treatment has greatly reduced the incidence of acute rejection, and facilitated early restoration of renal function (serum creatinine level less than 2.0 mg/dl within 1 week after transplantation). In patients with such good renal function, MZR is excreted rapidly in urine, so sufficient immunosuppressive effect may sometimes not be obtained at the currently approved dose.

To clarify the state of MZR use in Japan, Akiyama et al. [8] surveyed patients who had received MZR in the early stage after renal transplantation at 21 institutions between January 1999 and June 2001, and reported the results for 140 patients who had received MZR together with FK. Using the daily dose of MZR as an index, patients were classified into groups: group 1, receiving less than 3 mg/kg ($n = 37$); group 2, receiving at least 3 mg/kg, but less than 5 mg/kg ($n = 63$); and group 3, receiving at least 5 mg/kg ($n = 40$). Dose efficacy and safety were then examined. Among these 3 groups, no significant differences were found in patient or graft survival rates. However, the acute rejection-free rate was 85.0 % in group 3, significantly higher than the 64.9 % in group 1 and 65.1 % in group 2. With regard to adverse reactions that developed in the first year after transplantation, incidences of elevated uric acid levels and hypertension were significantly higher in group 3, while incidences of CMV and herpes zoster infection were not different in group 3 from those in the other groups.

On the basis of these results, for the purpose of examining the safety of high doses (phase 1), a single-dose study of MZR at doses up to 12 mg/kg/day and a 7-day consecutive administration study at a daily dose of 12 mg/kg (divided dose of 6 mg/kg in the morning and afternoon) were conducted in the UK. That study found barely any adverse reactions attributable to MZR, suggesting the safety of this dosage [3].

On the basis of all the above previous findings, we conducted the present multi-center cooperative randomized comparative study of MMF, the current international standard as a metabolic antagonist for renal transplant recipients, and conducted comparisons with high-dose MZR. We found that patient and graft survival rates in the first year after transplantation were 100 % in both groups, with no significant inter-group difference in the acute rejection-free rate. These findings suggest that high-dose MZR was as effective as MMF.

With regard to adverse events, many patients in the MZR group showed elevated uric acid levels, while many in the MMF group developed infections and digestive organ disorders, suggesting that each drug induced characteristic adverse events. Suppression of CMV proliferation by MZR in vitro has recently been reported [9], and this action of MZR appeared to contribute to the lower rate of CMV infection in the MZR group.

When considering both treatment methods from a medical economical aspect, the drug price of MZR 600 mg becomes about 1.3 times higher than that of MMF 2 g. However, curative drugs were administered in only 2 CMV infection-free recipients among the 4 recipients that developed CMV viremia in the MZR group while drugs were administered in all 8 recipients who developed CMV viremia in the MMF group. Furthermore, 2 of them had to be hospitalized because they developed infection. Taking this into consideration, it seems that the medical economy is almost the same. The results of this study seem to be significant in a sense that it increases the therapeutic options.

Since basiliximab was not used in the immunosuppressive protocol for the present study, the rejection rate was high in both groups. We therefore judged that further entry of new patients into this study was scientifically invalid, and concluded the study before reaching the target number of patients. On the basis of the accumulated results, we are

now conducting a multi-center cooperative randomized comparative study of 4 drugs in combination with the addition of basiliximab, and comparing the efficacy of MZR and MMF. Further investigations will be aimed at the establishment of better immunosuppressive therapies for renal transplant patients.

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Conflict of interest None.

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